Lab Notebook:
Cellulose Project

7/6/2015- 7/10/2015

1. Grew culture of *G. hansenii* in medium
2. Still growing for the entire week

7/13/2015 – 7/17/2015

1. Culture still growing
2. Read more about cellulose binding domain

7/20/2015 – 7/24/2015

1. Designed the cellulose binding system – originally plan to do antibody design. Reason is that the antibody can bind to one specific region while chemically activated streptavidin can bind to multiple regions of the proteins and can inhibit the function of the protein.
    a. Cons: The sequence for the Flag tag antibody is not sequenced thoroughly meaning that there are high risk the design will not work just because the sequence is wrong.

Antibody Design:

T7 - rbs - pel B - ATG - sfGFP - linker - CBD- linker - VH - linker - VL - (His)6 tag - Terminator
T7 promoter - rbs - sfGFP - linker - CBD - linker - Pel B - ATG - Vh - Linker - Vl - (His)6Tag - Terminator

711 bp + 700 +
ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCC + ATG +
aatgctacgccaactaagggtgcaaccccgaccaacacagcaacgcctacaaaaagcgctacagcaacacctacaagaccgtcagttcctacaaac
acac
cgactaacacaccggcaaatacacctgtttcaggcaacttgaaggtc gaGttc
tataactcaaatccgagtgatacaactaacagtattaatccgcagtt
taaagtaacaaatacaggatcaagtgcaattgatctttcaaagcttacattgagatactattacaccgttgatggccagaaggaccagactttctggtg
t
gaccatgcagctatcataggtagcaacggctcatacaacggcatcacatcaaatgtaaaaggaacattcgtaaagatgagctcaagcacaaataac
gcag
acacatacctcgaaataagtttcacaggtggcactttggaacctggtgctcatgtacagatacagggtaggtttgcgaaaaatgactggagtaattata
c
acagtcaaatgattactcatttaagtcagcatcacagttcgtagaatgggatcaggttacagcatatttgaatggagtacttgtatggggtaaagaacc
a
ggaggatcagtagttccgtcaacacagccggtaacaaccccaccggcaacaaccaagccgccagcaacaaccaaaccaccggctaccacgattcct
ccat
cagacgatccg + VH + linker + VL + (His)6 Tag

taatacgactcactatagggagatactagagaaagaggagaaatactagatggccggc +
ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCC
+ ATG +
cgtaaaggcgaagagctgttcactggtgtcgtccctattctggtggaactggatggtgatgtcaacggtcataagttttccgtgcgtggcgagggtgaa
g
gtgacgcaactaatggtaaactgacgctgaagttcatctgtactactggtaaactgccggtaccttggccgactctggtaacgacgctgacttatggtgt
tcagtgctttgctcgttatccggaccatatgaagcagcatgacttcttcaagtccgccatgccggaaggctatgtgcaggaacgcacgatttcctttaag
gatgacggcacgtacaaaacgcgtgcggaagtgaaatttgaaggcgataccctggtaaaccgcattgagctgaaaggcattgactttaaagaagac
ggca

atatcctgggccataagctggaatacaattttaacagccacaatgtttacatcaccgccgataaacaaaaaaatggcattaaagcgaattttaaaattcg
ccacaacgtggaggatggcagcgtgcagctggctgatcactaccagcaaaacactccaatcggtgatggtcctgttctgctgccagacaatcactatctg
agcacgcaaagcgttctgtctaaagatccgaacgagaaacgcgatcatatggttctgctggagttcgtaaccgcagcgggcatcacgcatggtatggatg
aactgtacaaa +
aatgctacgccaactaagggtgcaaccccgaccaacacagcaacgcctacaaaaagcgctacagcaacacctacaagaccgtcagttcctacaaacacac
cgactaacacaccggcaaatacacctgtttcaggcaacttgaaggtcgaattctataactcaaatccgagtgatacaactaacagtattaatccgcagtt
taaagtaacaaatacaggatcaagtgcaattgatctttcaaagcttacattgagatactattacaccgttgatggccagaaggaccagactttctggtgt
gaccatgcagctatcataggtagcaacggctcatacaacggcatcacatcaaatgtaaaaggaacattcgtaaagatgagctcaagcacaaataacgcag
acacatacctcgaaataagtttcacaggtggcactttggaacctggtgctcatgtacagatacagggtaggtttgcgaaaaatgactggagtaattatac
acagtcaaatgattactcatttaagtcagcatcacagttcgtagaatgggatcaggttacagcatatttgaatggagtacttgtatggggtaaagaacca
ggaggatcagtagttccgtcaacacagccggtaacaaccccaccggcaacaaccaagccgccagcaacaaccaaaccaccggctaccacgattcctccat
cagacgatccg + VH + linker + VL + (His)6tag - Terminator

T7 promoter - rbs - sfGFP - linker - CIP - linker -
taatacgactcactatagggagatactagagaaagaggagaaatactagatggccggc
cgtaaaggcgaagagctgttcactggtgtcgtccctattctg
gtggaactggatggtgatgtcaacggtcataagttttccgtgcgtggcgagggtgaaggtgacgcaactaatggtaaactgacgctgaagttcatctgta
ctactggtaaactgccggtaccttggccgactctggtaacgacgctgacttatggtgttcagtgctttgctcgttatccggaccatatgaagcagcatga
cttcttcaagtccgccatgccggaaggctatgtgcaggaacgcacgatttcctttaaggatgacggcacgtacaaaacgcgtgcggaagtgaaatttgaa
ggcgataccctggtaaaccgcattgagctgaaaggcattgactttaaagaagacggcaatatcctgggccataagctggaatacaattttaacagccaca
atgtttacatcaccgccgataaacaaaaaaatggcattaaagcgaattttaaaattcgccacaacgtggaggatggcagcgtgcagctggctgatcacta
ccagcaaaacactccaatcggtgatggtcctgttctgctgccagacaatcactatctgagcacgcaaagcgttctgtctaaagatccgaacgagaaacgc
gatcatatggttctgctggagttcgtaaccgcagcgggcatcacgcatggtatggatgaactgtacaaa accggc
aatgctacgccaactaagggtgcaa
ccccgaccaacacagcaacgcctacaaaaagcgctacagcaacacctacaagaccgtcagttcctacaaacacaccgactaacacaccggcaaatacacc
tgtttcaggcaacttgaaggtcgaattctataactcaaatccgagtgatacaactaacagtattaatccgcagtttaaagtaacaaatacaggatcaagt
gcaattgatctttcaaagcttacattgagatactattacaccgttgatggccagaaggaccagactttctggtgtgaccatgcagctatcataggtagca
acggctcatacaacggcatcacatcaaatgtaaaaggaacattcgtaaagatgagctcaagcacaaataacgcagacacatacctcgaaataagtttcac
aggtggcactttggaacctggtgctcatgtacagatacagggtaggtttgcgaaaaatgactggagtaattatacacagtcaaatgattactcatttaag

tcagcatcacagttcgtagaatgggatcaggttacagcatatttgaatggagtacttgtatggggtaaagaaccaggaggatcagtagttccgtcaac
ac
agccggtaacaaccccaccggcaacaaccaagccgccagcaacaaccaaaccaccggctaccacgattcctccatcagacgatccg

sfGFP: (711 bp)
cgtaaaggcgaagagctgttcactggtgtcgtccctattctggtggaactggatggtgatgtcaacggtcataagttttccgtgcgtggcgagggtgaa
g
gtgacgcaactaatggtaaactgacgctgaagttcatctgtactactggtaaactgccggtaccttggccgactctggtaacgacgctgacttatggtgt
tcagtgctttgctcgttatccggaccatatgaagcagcatgacttcttcaagtccgccatgccggaaggctatgtgcaggaacgcacgatttcctttaag
gatgacggcacgtacaaaacgcgtgcggaagtgaaatttgaaggcgataccctggtaaaccgcattgagctgaaaggcattgactttaaagaagac
ggca
atatcctgggccataagctggaatacaattttaacagccacaatgtttacatcaccgccgataaacaaaaaaatggcattaaagcgaattttaaaatt
cg
ccacaacgtggaggatggcagcgtgcagctggctgatcactaccagcaaaacactccaatcggtgatggtcctgttctgctgccagacaatcactatc
tg
agcacgcaaagcgttctgtctaaagatccgaacgagaaacgcgatcatatggttctgctggagttcgtaaccgcagcgggcatcacgcatggtatgga
tg
aactgtacaaa

linker - Cipa - linker
aatgctacgccaactaagggtgcaaccccgaccaacacagcaacgcctacaaaaagcgctacagcaacacctacaagaccgtcagttcctacaaac
acac
cgactaacacaccggcaaatacacctgtttcaggcaacttgaaggtcgaattctataactcaaatccgagtgatacaactaacagtattaatccgcag
tt
taaagtaacaaatacaggatcaagtgcaattgatctttcaaagcttacattgagatactattacaccgttgatggccagaaggaccagactttctggtg
t
gaccatgcagctatcataggtagcaacggctcatacaacggcatcacatcaaatgtaaaaggaacattcgtaaagatgagctcaagcacaaataac
gcag
acacatacctcgaaataagtttcacaggtggcactttggaacctggtgctcatgtacagatacagggtaggtttgcgaaaaatgactggagtaattata
c
acagtcaaatgattactcatttaagtcagcatcacagttcgtagaatgggatcaggttacagcatatttgaatggagtacttgtatggggtaaagaacc
a
ggaggatcagtagttccgtcaacacagccggtaacaaccccaccggcaacaaccaagccgccagcaacaaccaaaccaccggctaccacgattcct
ccat
cagacgatccg

PelB Sequence:
ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCCAGCCGGCGATGGCC

heavy chain: domain 3 1-118
evqlqqsgge lakpgasvkm sckssgytft ayaihwakqa agaglewigy iapaagaaay
61 naafkgkatl aadkssstay maaaaltsed savyycaraa aagadywgqg ttltvssak

Linker is GGGGSGGGGSGGGGS

light chain: 2-111 out of 216 aa
vlmtqaplt lpvslgdqas iscrssqaiv hangntylew ylqkpgqspa lliykvanrf
61 sgvpdrfsgs gsgtdftlki srveaedlgv yycfqgahap ytfgggtkle i

domain 1: 1-114
dvlmtqaplt lpvslgdqas iscrssqaiv hangntylew ylqkpgqspa lliykvanrf
61 sgvpdrfsgs gsgtdftlki srveaedlgv yycfqgahap ytfgggtkle ikra

His6 tag:

flag tag: DYKDDDK

anti-Flag M2

2. **Design of the streptavidin-biotin construct**: The streptavidin construct has one of the strongest binding affinity with biotin. Further research shows that bira can allow biotinylation of a specific peptide acceptor region known commercially as the Avitag sequence. The design of the construct is detailed below:

Streptavidin-Biotin Construct:

(1) CIPA - Streptavidin - terminator
(2) Pro - RBS - AP Tag - Protein of Interest - end
(3) Pro - RBS - BirA - end

BIRA - in cytoplasm expressed from http://www.uniprot.org/uniprot/P06709.fasta
MKDNTVPLKLIALLANGEFHSGEQLGETLGMSRAAINKHIQTLRDWGVDVFTVPGKGYSL
PEPIQLLNAKQILGQLDGGSVAVLPVIDSTNQYLLDRIGELKSGDACIAEYQQAGRGRRG
RKWFSPFGANLYLSMFWRLEQGPAAAIGLSLVIGIVMAEVLRKLGADKVRVKWPNDLYLQ
DRKLAGILVELTGKTGDAAQIVIGAGINMAMRRVEESVVNQGWITLQEAGINLDRNTLAA
MLIRELRAALELFEQEGLAPYLSRWEKLDNFINRPVKLIIGDKEIFGISRGIDKQGALLL
EQDGIIKPWMGGEISLRSAEK

Avitag-Can be at the N or C terminus
GLNDIFEAQKIEWHE
GLNDIFEAQKIEWHE
if fused at the N terminus, need a methionine start coon with a second codon
consistent with efficient translation and protein stbility.
starting MA seuence, ATGGCT,

85-11 tag: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2144313/pdf/10211839.pdf
MAGGLNDIFEAQKIEWHE

At C-terminus, the 15-mer is connected to the protein through a minimum a GG peptide
linker

Streptavidin : Part BBa_J36841
gctgaagctggtatcaccggcacctggtacaaccagctgggatccaccttcatcgttaccgctggtgctgacggtgctctgaccggtacctacgaatcc
g
ctgttggtaacgctgaaagccgctacgttctgaccggtcgttacgactccgctccggctaccgacggttccggaaccgctctgggttggaccgttgcttg
gaaaaacaactaccgtaacgctcactccgctaccacctggtctggccagtacgttggtggtgctgaagctcgtatcaacacccagtggttgttgacctc
c

ggcaccaccgaagccaacgcgtggaaatccaccctggttggtcacgacaccttcaccaaagttaaaccgtccgctgcttcccatcaccatcaccacca
tt
aataa

7/27/2015 – 7/31/2015

1. Retrieved the cellulose
2. Looked into ways to process cellulose into finer sheet
    a. Tina used DIY paper-making protocol to do cellulose binding
3. Looked into ordering the sequence for two constructs:

CIPA: T7 promoter – RBS – CIPA CBD – Monomeric Streptavidin – End
BirA: J23104 promoter – RBs – AP Tag (Acceptor Peptide) – SgfI – aeBlue (Protein of Interest) - Pmei–
RBS – biotin ligase – End

8/3/2015 – 8/7/2015

1. IDT synthesis arrived:
2. PCR cipA and birA
3. Digested and ligated into psb1C3 plasmid
4. Transformation of cipA and birA

8/10/2015 – 8/14/2015

1. Too many red colonies for cipA and birA: understandable because use RFP backbone
2. Picked out white colonies for overnight culture
3. cipA white colonies were actual gene; however, birA failed
4. Re-PCR and transformed of birA gene

8/17/2015 – 8/21/2015

1. Re-ligate and transformed with using CRATER method
    a. Successful at removing most of the red colonies from using RFP as the backbone plasmid
    b. However, the aeblue was not present
2. Colonies PCR and sequencing show that birA still is not there
3. Looked at geneious file for the gene sequence and realized that there is a PstI site at the birA gene.
4. Planned to use E and S as digestion enzyme instead of E and P

8/24/2015 – 8/28/2015

1. Digested with E and S digestion for birA; ligated and transformed
2. Sequencing did not work.
3. Couldn't figure out the problem

8/31/2015 – 9/4/2015

1. Tried to do digestion and ligation again but could not work
2. Looked into Gibson Assembly and tried Gibson
3. Sequencing after Gibson assembly still failed

9/7/2015 – 9/11/2015

1. Retried Gibson Assembly but still failed
2. Also did autolysis cloning and the autolysis cloning works successfully!

9/14/2015 – 9/18/2015

1. PCR the IDT Bira Gibson and send for sequencing
2. Sequencing of the PCR failed
3. This explained the reason that sequencing keeps failing
4. Did preliminary protein extraction of birA and cipA.
    a. There are bands for cipA and birA; however, it does not match the right size of cipA and birA
    b. Other protein extraction done at the same time failed. Possible reason for failure of cipA is that the flag-tag affinity does not work
5. Will redo protein extraction of cipA and re-clone birA